Phenotype analysis of tumor cells with eight color FISH

Yuko Ito[≠], Jingly Fung[≠″], Joanne Hsu[≠], Nir Katzir[≥], Robert A Lersch[≠], and Heinz-Ulrich G. Weier[≠]

[≠] Life Sciences Division, University of California, Lawrence Berkeley National Laboratory, Berkeley, CA 94720, USA, [″] Reproductive Genetics Unit, Department of Obstetrics, Gynecology and Reproductive Sciences, University of California, San Francisco, CA 94143-0720, USA, [≥] Applied Spectral Imaging, Migdal HaEmek 10551, Israel.

High throughput gene expression profiling using cDNA microarrays generates a wealth of information and often demonstrates tumor-specific changes. These measurements, however, provide average values for tumor cell populations that may be rather heterogeneous. Our technical developments address the issue of heterogeneity in tumor research by developing an analytical system capable of performing semi-quantitative multi-gene expression profiling of single cells. Targeting cell-by-cell measurements of expression levels of multiple tumor markers, our approach uses RNA/cDNA fluorescent in situ hybridization (FISH) combined with Spectral Imaging and digital image analysis. While the system is capable of deconvoluting images of objects stained with up to nine fluorochromes, we performed initial tests of system resolution and reproducibility with commercially available beads fluorescing in seven different wavelength intervals. The system measured up to our expectation of being able to quantitate the seven different fluorescent reporter molecules with relative standard deviations ranging from 1% to 6.1%. Using eight different fluorochroms, we then analyzed the expression levels of 6 different tyrosine kinase gene and one genomic target in breast and thyroid cancer cells counterstained with DAPI. In artificial mixtures, the system was able to recognize the tumor cells based on the level of expression of one or two genes, and could identify cells present in only a few percent. Supported by NIH grants CA88258 and CA80792 and the United States Army Medical Research and Material Command, United States, Department of the Army (DAMD17-99-1-9250, DAMD17-00-1-0085).